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Effect of gamma irradiation on microbiological and physicochemical properties of carrot (*Daucus carota l.)* and waraka (*Artocarpus heterophyllus l.)* in Sri Lanka.

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Abstract
Waraka and Carrot are in high demand locally and globally export market as key food industry ingredients. This study evaluated the effects of different gamma irradiation (0, 2, 4, 6, 8, 10 kGy) on their microbial, chemical, and physical qualities to promote it as a preservation technique. Irradiation using (Co-60) source at a dose rate of 5.3 Gy/min was applied. Microbial parameters such as total plate count, yeast, mold, and coliform counts were assessed. Total Plate Counts and yeast and mold counts were dropped significantly with increased doses, while E. coli were not found. Chemical analysis indicated no impact on antioxidant and phenolic contents, although beta-carotene decreased at higher doses. Physical parameters such as moisture content and water activity were also measured. Optimal doses of 2kGy and 4 kGy were identified to preserve nutritional, physical, and microbial quality, for Waraka and carrot, ensuring extended shelf life and safety.

Keywords: Carrot, dose, gamma irradiation, microbial safety, waraka

1. Introduction
Carrots (*Daucus carota L.*) stand as a globally significant root crop, renowned for their rich content of β-carotene, B vitamins, fiber, and essential minerals, as highlighted by Prakash in their study (Prakash, Upadhyay, Pushpangadan & Gupta, 2011) [16]. Their consumption is associated with various health benefits, including reduced cholesterol levels, lowered risk of high blood pressure, stroke, heart disease, and certain types of cancers and Abu-Khalaf, Bennedsen & Bjorn (2004) [1] note that carrots are commonly prepared either alone or in combination with other vegetables in dishes such as soups, stews, curries, and pies mostly in food processing industry. The widespread cultivation of carrots has led to the development of dehydration processes, driven by the demand from the instant noodles industry and their versatility in various recipes. Consequently, carrot powder and chips have emerged as prominent ingredients in diverse cuisines. Post-harvest wastage during the peak period of carrot cultivation is very high. These wastages are due to microbial infestation, improper post-harvest handling, lack of marketing, transportation and storage facilities etc. So, attempt was made to preserve this valuable root crop to reduce post-harvest loses by dehydration to study the shelf life (Gani, Bhat & Beenish. 2018) [8].
Jackfruit (Artocarpus heterophyllus L.) also known as “Waraka” in Sri Lanka. Jackfruit is an important fruit, extensively cultivated in tropical, subtropical, and temperate regions of the world (Arora & Parle, 2016) [3]. Jackfruit demand has been gradually increasing, both domestically and in export markets (Nakintu, Olet, Andama & Lejju, 2019) [14]. The fruit and seeds are rich sources of minerals, vitamins, organic acids, and dietary fiber. Previous research has shown that jackfruit has anticarcinogenic, antimicrobial, antifungal, anti-inflammatory, wound healing, and hypoglycemic properties, all of which can be attributed to its diverse nutrient and biochemical profile (Ranasinghe, Maduwanthi & Marapana, 2019) [17].
Since jackfruit is highly perishable, processing is needed to preserve the fruit and reduce postharvest losses. Minimal processing techniques, refrigeration, and dehydration or drying are among the useful processes used to preserve jackfruits (Anaya *et al*., 2018) [2].
Irradiation serves as a preservation technique involving the exposure of food to radiation. This method is considered a safe, wholesome, and hygienic technology employed within the food industry (Indiarto & Qonit, 2020) [11].

Irradiation method encompasses subjecting food items to ionizing radiation prior to packaging or in substantial quantities, effectively diminishing the risks associated with foodborne illnesses while preventing or arresting processes such as budding or ripening (Ashraf, Sood, Bandral, Trilokia & Manzoor, 2019) [4].

Irradiation induces harm to a cell's genetic material through DNA lesions or the cleavage of both DNA strands. This disruption inhibits replication, leading to the arbitrary suppression of cellular functions and ultimately culminating in cell demise (Lacroix & Follett, 2015) [13].

1.1 Problem statement

Microorganism growth is common in fruit & vegetables and spices at various stages of processing, including cultivation and harvesting, making them potential food contaminants. Ntuli *et al*., (2017) [15] found that over half of the samples analyzed were unsuitable for human consumption due to poor microbial quality, falling below international standards. This highlights the public health risks posed by pathogens such as E. coli, coliform bacteria, yeast, and molds in dehydrated fruits and vegetables. Effective techniques, such as gamma irradiation, can be applied to reduce microbial load while preserving quality attributes of commodity (Ntuli *et al*., 2017) [15].

In Sri Lankan scenario carrot and waraka are the two high demanded fruit and vegetable in food industry and for the time being dehydration technique is applied as a preservation technique for such type of products. Food irradiation little bit new era for Sri Lankan food industry and the main objectives of this study were to be revealing the gamma irradiation of food items as an effective preservation technique for carrots and Waraka, and to assess the microbial safety of both commodity while preserving other quality attributes.

2. Materials and Methods

2.1 Analysis of microbial parameters

Total Plate count (TPC) (ISO 4833-1: 2013), yeast and mold count (ISO 21527-2: 2008) and coliform tests (ISO 4831: 2006) of Waraka and Carrot samples were determined.

As reagents for TPC, Buffered peptone, plate count agar, incubator (memmert ICP600), oven (memmert ICP600), laminar air flow (FASTER Bio48), autoclave (SA-300VF), colony counter (Galaxy 330), stomacher blender (400CC), glass wares (petri plates, conical flasks, pipettes).

Microbial analysis began with a 10^{-1} dilution in buffered peptone, followed by serial dilutions. 1 ml of each dilution was plated on sterile plate count agar and incubated at 30°C for 48 hours. Colony-forming units (CFU) were counted on plates with 30 to 300 colonies, providing a quantitative assessment of microbial presence.

$$\frac{\text{CFU}}{\text{g}} - 1 \quad = \quad (\text{Number of colonies} \times \text{Volume of dilute suspension})/(\text{Dilution factor})$$

Yeast and molds count were analyzed by using ISO 21527-2: 2008 modified method.

The microbial analysis began with a 10^{-1} dilution using 90 ml of buffered peptone and 10g of the sample. Subsequently, dilution series were prepared, and 0.1 mL of each dilution was plated on PDA and incubated at 25 °C for 5-7 days. CFU were counted on plates with 15-150 colonies

Total coliform, fecal coliform and E. coli Coliforms were analyzed by using ISO 4831: 2006 modified method.

In the initial phase, 90 ml of buffered peptone served as the diluent, with 10 g of the sample added and thoroughly mixed. Subsequently, a 10-1 dilution was prepared by adding 1ml of the sample to 9 ml of buffered peptone, followed by thorough mixing, and this process continued to create a dilution series.

MacConkey broth was then prepared and dispensed into test tubes with Durham tubes inserted. Prepared dilutions were added to these tubes, which were then incubated at 37 °C for 24 hours in a water bath.

In the confirmation stage, after the incubation period, growth indicating gas production and a color change from purple to yellow were observed. Tubes showing gas production were designated as positive, and the number of positive tubes at each dilution was recorded.

Positive samples were further inoculated into Brilliant Green Bile Broth and incubated at 37 °C for 24 hours in a water bath to identify total coliforms. Simultaneously, another set of samples was placed at 44 °C for 24 hours to identify fecal coliforms.

In the completion phase, positive tubes exhibited growth with gas production, and the number of positive tubes was noted. Subsequently, the total coliform and fecal coliform counts were determined.

Fecal coliform tubes were then subjected to an E. coli test using eosin methylene blue agar medium and incubated at 30°C for 24 hours. The presence of E. coli was confirmed by the appearance of greenish metallic sheen characteristic colonies.

2.2 Analysis of Chemical parameters

Plant extraction was performed using a sonicator extraction method for both samples. Initially, 10 g of each sample was measured and blended to achieve size reduction. The blended samples were then placed in a freeze dryer to remove moisture. Following this, the samples were mixed with 70% ethanol at a 1:10 ratio. The mixture was sonicated at 30°C for 15 minutes to obtain the extract. This process was repeated three times to maximize extraction. Finally, the filtrate was collected by filtering the extract through filter paper.

The total phenolic content was measured calorimetrically using the folin- Ciocalteu (FC) method for the irradiated and non-irradiated waraka samples.

The total phenolic content was determined using the Folin-Ciocalteu (FC) method. Anhydrous gallic acid was dissolved to prepare a 100 mg/L stock solution, from which standard concentrations were made to create a calibration curve. Plant extracts (200 µL) were mixed with 1 mL of ten-fold diluted Folin-Ciocalteu reagent and allowed to react for 8 minutes. Then, 800 µL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated for 1 hour. Absorbance was measured at 765 nm using a UV spectrophotometer.

Beta carotene content was measured by using AOAC 1980 method for irradiated and non-irradiated carrot samples.

Beta-carotene content was determined using the AOAC 1980 method. Carrot extracts were mixed with hexane and acetone (1:2 ratio) in a separation funnel. The mixture was

swirled gently to obtain a homogenous mixture. The bottom layer was run off into a beaker and the top layer was collected into conical flask and. The extraction was repeated 5–6 times until the extract became fairy yellow. The final extract was analyzed at 436 nm using a UV spectrophotometer.

The beta-carotene concentration was calculated as.

$$\text{Concentration of beta carotene} = \text{"Absorbance"} / (\text{"Extinction coefficient} \times \text{thickness of cuvettes}$$

2.3 Analysis of physical parameters

Water activity and Moisture content of Waraka and Carrot were analyzed. Each analysis was performed in triplicate.

Water activity was measured by using a Novasina Lab Master Water activity meter at 25 °C. For moisture content determination, 1g of each sample was evenly distributed in aluminum dishes and heated at 105±1°C with a moisture analyzer following the modified AOAC 925.10 method.

3. Results and Discussion

3.1 Microbial parameters

According to the figure 01, the highest mean total plate count was observed in the control sample of Waraka ($1.5\times10^6\pm3.0\times10^5$ CFU/g) and the lowest was observed in the 10 kGy sample (0.00±0.00 CFU/g) sterile condition.

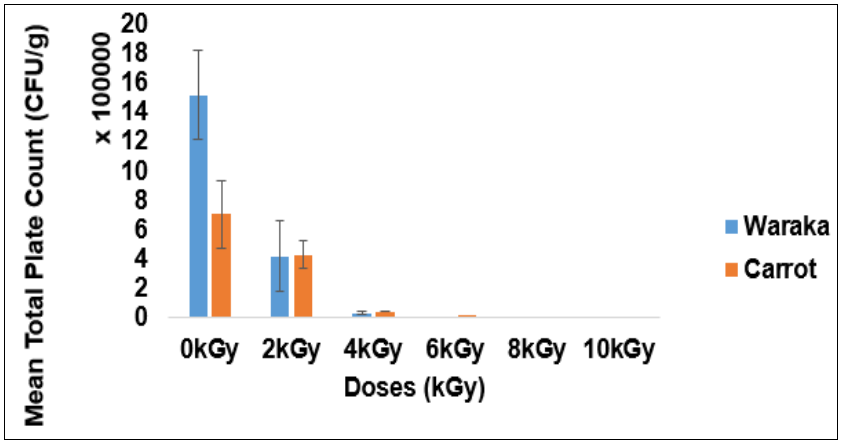

Fig 1: Total Plate Count of Waraka and carrot samples at different irradiated doses

The study examined the total plate count (TPC) in irradiated carrot samples, revealing significant differences. The control sample exhibited the highest TPC ($7.0\times10^5\pm2.3\times10^5$ CFU/g while the 10kGy sample showed the lowest (0.00±0.00 CFU/g). Analysis using the Kruskal-Wallis Test confirmed a substantial reduction in TPC due to irradiation ($p<0.05$), with significant distinctions among the six doses. Notably, irradiation led to a drastic decrease in TPC, rendering the 8kGy and 10kGy samples sterile.

According to this study, jackfruit contain high amount of microbial count. Since the fleshy nature and high nutrition content, it might be highly contamination from different pathogenic microorganisms. However, with the increment of the irradiation doses microbial count get decreases

Carrot is a perishable fruit which microorganisms can grow easily. According to the Ghosh and Ganguli study, there is a difference microbial load in the different point of distribution chain. Total viable and Staphylococcus counts showed a significant increase from 10^4 to 2.5x 10^6 CFU/ g and 1.9 x 10^4 to 7.7 x 10^6 CFU /g respectively during its transit from central distribution site to street vendors (Ghosh, Ganguli, & Mudgil, 2004) [9].

In Figure 2, the yeast and mold counts are depicted, with the control sample having the highest count. However, as irradiation doses increased, there was a significant reduction in yeast and mold, with sterilization achieved at 6 kGy, 8 kGy, and 10 kGy.

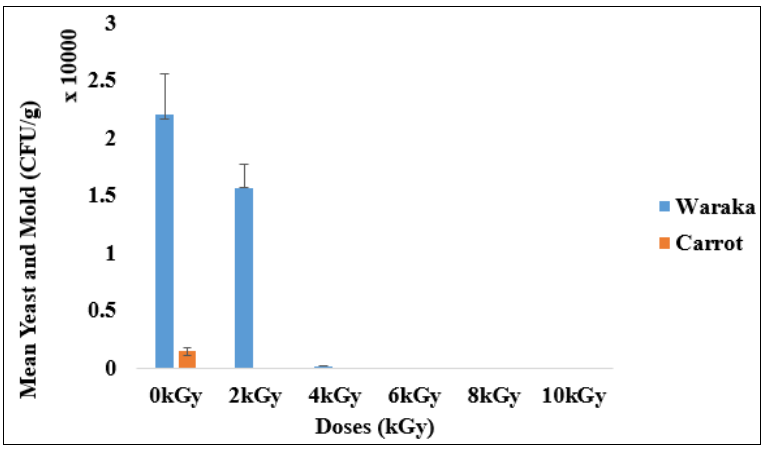

Fig 2: Yeast and mold Count of Waraka and carrot at different irradiated doses

The sample medians for the six treatments were calculated. According to the Kruskal-Wallis Test, there is a significant reduction of yeast and mold with increasing the irradiation doses (p<0.05) (Kruskal-Wallis Test DF=5, 18, P= 0.012). After 2 kGy irradiation dose, the yeast and mold count were dramatically reduced in both samples. 6 kGy, 8 kGy and 10 kGy is the sterile condition.

The highest Yeast and Mold count was observed in the control sample of waraka and carrot ($2.2\times10^4\pm3.6\times10^3$ CFU/g) and ($1.4\times10^3\pm3.0\times10^2$ CFU/g) respectively.

According to the research of Shwetha and B. Ranganna, during storage study of jackfruit squash samples, the squash was observed at monthly intervals for bacteria, yeast and moulds growth. The processed jackfruit squash from different genotypes were subjected to microbial analysis by employing Dilution Plate Count Method (Shwetha & Ranganna, 2018) [18]. Microorganisms, typically residing on fruit and vegetable surfaces, can penetrate inner tissues through surface damage, as noted by ghosh's study yeast and mold counts in fresh and peeled Carrots, with variations based on Carrot type (Ghosh, Ganguli, & Mudgil, 2004) [9].

The findings suggest that gamma irradiation can effectively enhance the microbiological quality of Carrots, making them suitable for domestic and export markets.

Figure 3 displays the total coliform count in irradiated and non-irradiated Waraka and carrot samples. The control sample had the highest count, while the 10 kGy sample showed the lowest count. Irradiation significantly reduced total coliform count, with a notable decline starting at 4 kGy and reaching nearly to zero.

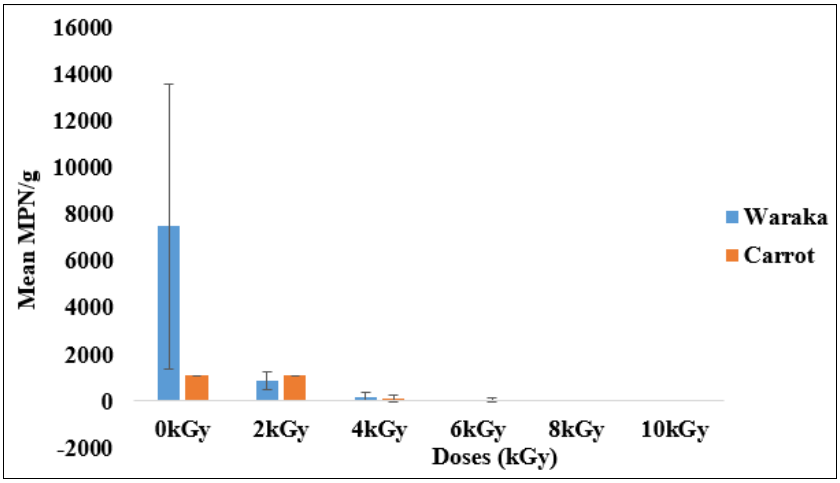

Fig 3: Total coliform count of Waraka and carrot sample at different irradiated doses.

The highest total coliform count was observed in control sample of Waraka ($7.4\times10^3\pm6.0\times10^3$ MPN/g) and lowest was observed in 10 kGy sample (0.30±0.00 MPN/g). With high irradiation doses total coliform count was dramatically decreased. The highest total coliform count was observed in control samples of carrot ($1.10\times10^3\pm0.00$ MPN/g) and lowest was observed in 10 kGy sample (0.00±0.00 MPN/g). With high irradiation doses total coliform count was dramatically decreased.

However fecal coliform and E.coli was not detected even from the control sample of both samples.

The findings reveal that controlled samples of dehydrated waraka exhibited contamination from bacteria, yeast, and mold. However, upon exposure to gamma radiation doses, a significant reduction in microbial load was observed.

3.2 Chemical parameter

Total phenolic content and beta carotene content of irradiated and non-irradiated Waraka and carrot samples were analyzed,

The presence of antioxidant content in jackfruit varies depending on the part of the tree. Jackfruit leaves exhibit the highest antioxidant content, followed by the seed flour, which shows a higher DPPH inhibition percentage compared to the bulb (Gokhale, Toro & Patwardhan, 2015) [10].

According to the table 01, the amount of phenolic concentration is slightly differ with the irradiation doses. The highest phenolic concentration was recorded in 0 kGy 16.36±0.533 mg GAE/100g and lowest phenolic concentration was recorded in 8 kGy sample 11.20±0.72 mg GAE/100g. There is a significant difference between the phenolic concentration and irradiation doses (p<0.05) (ANOVA DF=5, 17, P= 0.00). The polyphenolic compounds reported in jackfruit include phenolic acids such as gallic acid, ferulic acid and tannic acid and flavonoids catechin, rutin and myricetin (Singh, Maurya, Mandawi, & Pratap 2015) [21].

Table 1: Phenolic concentration of Waraka in different irradiated doses

Doses (kGy)	Phenolic concentration (mg GAE/100g)
0 kGy	16.36±0.533
2 kGy	13.94±1.11
4 kGy	11.98±0.23
6 kGy	11.63±0.51
8 kGy	11.20±0.72
10 kGy	11.92±1.6

Raw jackfruit skin has the highest total phenolic content with respect to the gallic acid standard 22.73±2.04 and ripe jackfruit have 12.08±1.03 µg/g fresh weight. And also ripe jackfruit flesh have the 19.31±1.8 µg/g of total phenolic content. ((Singh, Maurya, Mandawi, & Pratap 2015) [21].

Figure 04 shows that, there's a significant decrease in beta carotene levels with increasing irradiation doses.

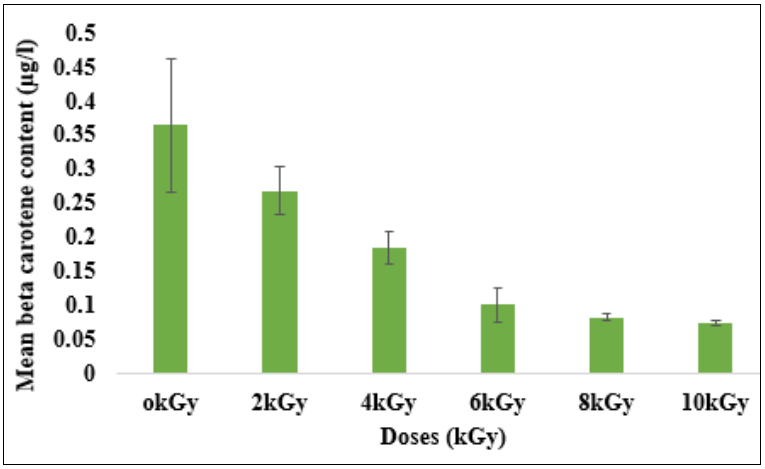

Fig 5: Beta carotene content in irradiated and non-irradiated sample of carrot

According to the study there is a significant difference between the beta carotene level and doses. (p<0.05) (ANOVA DF=5, 17, P= 0.00). The highest beta carotene level is 0.363±0.077 µg/L and recorded the highest beta carotene level in control sample and lowest beta carotene level was recorded in 10 kGy sample and it was 0.073±0.003 µg/L.

The total carotenoids content in the edible portion of carrot roots range from 6,000 to 54,800 µg/100 g (Simon & Lindsay, 1983). According to the findings in the past decade carotenoids such as β-carotene have attracted considerable attention because of their possible protective effect against some types of cancers (Bast, van der Plas, van den Berg & Haenen, 1996) [6]

This study shows however reduction of Beta carotene level with respect to the radiation doses and however microbial effect level; of 2Kgy will not show the significance reduction of beta carotene level with respect to the control sample.

Beta carotene is a type of antioxidant and content of antioxidant in carrot can be varying with the different extraction solvent. Methanol extracted has the highest antioxidant content and ethanol extracted antioxidant content is lower than the methanol extracted sample. Methanol extract has 46788±200.88 µmol/g and ethanol extract have 32303±200.91 µmol/g (Shyamala & Jamuna, 2010) [19]. According to the study of Boadi *et al*., (2021) [7] antioxidant content may vary with the varieties of carrot and the Carrot extracts showed high antioxidant activities and recorded mean peroxide scavenging values of 80.10±8.35%, 83.27±9.04%, 86.28±7.64%, respectively, for Pamela, Kuroda, and Amazonia.

3.3 Physical parameters

Moisture content of Waraka samples were varied from 96.400%-97.72%. According to the study highest mean moisture content of Waraka sample was observed in the 4kgy sample (97.85±0.29) and the lowest was observed in 6kgy sample (96.45±0.11). The moisture contents of the irradiated Waraka samples were significantly higher (p<0.05) compared to the control. There is a significant difference between 4kgy, 8kgy and 10kgy irradiated samples (ANOVA, DF=5, 17, p=0.00). According to this study, the moisture content of carrot samples was varied from 95.50%-97.19%. According to the study highest mean moisture content of carrot sample was observed in the 4 kgy sample (97.10±0.12) and the lowest was observed in 10 kgy sample (95.59±0.12). The moisture contents of the irradiated carrot samples were significantly higher (p<0.05) compared to the control. There is a significant difference between 4 kgy, 8 kgy and 10 kgy irradiated samples (ANOVA, DF=5, 17, p=0.00).

In this study water activity of waraka samples were varied from 0.478-0.511. According to the the highest mean water activity was observed in 4 kgy sample (0.508±0.001) and the lowest water activity was observed in 10kgy sample (0.48±0.002). The water activities of irradiated waraka samples were significantly lower (p<0.05) compared to the control. Water activity of 4kgy sample, 8kgy sample and 10 kgy sample ware significantly different (anova, DF=5, 17, p=0.00).

In this study water activity of carrot samples were varied from 0.481-0.511. According to the study highest mean water activity was observed in 0kgy sample (0.507±0.001) and the lowest water activity was observed in 10kgy sample (0.48±0.002). The water activities of irradiated carrot samples were significantly lower (p<0.05) compared to the control. Water activity of 4kgy sample, 8kgy sample and 10 kgy sample ware significantly different (anova, DF=5, 17, p=0.00).

Conclusion

Gamma irradiation is a preservation technique that can be successfully applied for food industry. According to that study, gamma irradiation does not cause significant changes in the physical parameters (moisture, water activity) and chemical parameters (antioxidant, phenolic content, beta carotene) of dehydrated Waraka sample and dehydrated Carrot sample. According to the results of the study, dehydrated Waraka contains a higher amount of microbial count than the dehydrated Carrot. A Drastic reduction in microbial count can be seen in 4 kGy in dehydrated Waraka and 2 kGy in dehydrated Carrot. The study shows 4 kGy and 2 kGy are the possible doses to eliminate the microbial contamination of dehydrated Waraka and Carrot respectively. This treatment can be used as safe and efficient method for preventing microbial spoilage while preserving the other physical parameters.

Future perspectives

Irradiation has proven highly effective against living organisms with DNA and/or RNA, while causing minimal

alterations in macronutrient content. Even at doses exceeding 10kGy, proteins, fats, and carbohydrates maintain their nutritional value, albeit with potential sensory changes. Essential amino acids, essential fatty acids, minerals, and trace elements remain largely unaffected. Some vitamins, primarily thiamine, may experience a decrease, but these changes are comparable to those observed in other manufacturing processes like drying or canning (Basbayraktar, Halkman, Yucel & Cetinkaya, 2006) [5]. To date, over 50 countries have granted approval for the irradiation of more than 60 foods and food products, either conditionally or unconditionally, with these numbers increasing annually. Commonly treated products include spices, dried herbs, and vegetable seasonings, with over 90,000 tons irradiated in 2000. For example, the irradiation of hamburgers in the USA surged from 6,800 tons in 2002 to over 22,000 tons in 2003 (Koopmans & Duizer, 2004) [12].

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